Molecular Diagnosis

In Oncology & Genetics

Diagnostic Molecular Pathology

- USE OF:
  - Sequence Specific INFORMATION
  - MACROMOLECULES
  - for:
  - Risk identification
  - Diagnosis
  - Prognosis
  - Prediction of response to therapy
  - Monitoring therapeutic responses

Macromolecules

- Peptides/proteins
- Polysaccharides
- Polynucleotides/nucleic acids

“Nucleic Acid Diagnosis”

- Use of specific sequence information
  - in nucleic acids
  - DNA and RNA
  - for clinical diagnosis

Analysis Of Information In Nucleic Acids

- Sequencing
- Hybridization
- Amplification
  - with specific primers
- Restriction enzyme digestion
  - Recognize specific sequences
- Electrophoretic mobility
- Translation

Molecular Oncology

- DIAGNOSTIC/PROGNOSTIC INFORMATION PROVIDED BY:
  - Gross alterations in DNA content of tumors
  - Cell cycle information
  - Molecular Markers of Clonality
  - Oncogene/Tumor Suppressor gene mutations
  - Tumor Specific Translocations
  - “Tissue specific” mRNA in tumor staging
  - Minimal residual disease determination
Identification Of Clonal Proliferations

- Antigen receptor gene rearrangements.
  - Southern Blotting: IgH, TCR; EBV termini.
  - PCR: Ig and TCR gene rearrangement.
- X-inactivation.
  - Human androgen receptor assay.
- Microsatellite allelotyping.

Translocations w/o gene fusion

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Translocation</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing's Sarcoma</td>
<td>(11;22); (12;22)</td>
<td>EWS/FLI1; EWS/ERG; EWS/EYT1; EWS/EYT4</td>
</tr>
<tr>
<td>Alveolar Rhabdomyosar.</td>
<td>(1;13); (2;13)</td>
<td>PAX7/FOXH1; PAX3/FOXH1</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>(1;18)</td>
<td>SYT/SSX1</td>
</tr>
<tr>
<td>DSRCT</td>
<td>(11;22)</td>
<td>EWS/WT1</td>
</tr>
<tr>
<td>Myxoid round cell liposarcoma</td>
<td>(12;22)</td>
<td>CHOP/FUS</td>
</tr>
<tr>
<td>Clear cell sarcoma soft parts</td>
<td>(12;22)</td>
<td>EWS/ATF-1</td>
</tr>
<tr>
<td>Extraskeletal myxoid chondrosar.</td>
<td>(19;22)</td>
<td>EWS/TEC</td>
</tr>
</tbody>
</table>
**TRANSLOCATIONS: DETECTION METHODS**

- **Fusion product:**
  - Detect at DNA or RNA level.
  - DNA level: FISH, Southern blotting.
  - RNA detection: RT-PCR
    - Highly sensitive.
    - Cheaper.
    - "Real-time" detection.
    - Semi-quantitative detection – minimal residual disease/quantification.
    - Chimeric transcript detectable during "complete remission": rising titer - impending relapse.
    - Need for fresh tissue (in general)

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- **RNA detection:** RT-PCR
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**Spectral Karyotyping (SKY)**

- FISH w/multiple probes to identify *all* chromosomes
  - Identify *any* translocations, markers etc. w/one test.
  - Need for special equipment
  - Need for metaphases.

**Gene Amplifications & Specific Mutations**

- **Amplification**
  - n-Myc: neuroblastoma.
  - Her2/Neu: breast cancer.

- **Mutations:**
  - C-Kit: gastrointestinal stromal tumors.
  - EGFR: Lung CA response to Iressa.
  - p53: poor prognosis, reduced chemosensitivity.

**Tumor Suppressor Gene Mutations**

- "loss of function mutations"
  - many possible mutations
  - "hot-spots"
    - e.g., p53: Exons 6, 7, 8, 9 > 90% of mutations
  - truncated protein: "protein truncation test"
  - "whole gene sequencing"
    - Tumor percentage.

**“Oncogene” Mutations**

- "gain of function mutations."
  - limited number for each gene.
    - "regulatory site mutations" - "constitutive activation."
    - "active site mutations" - "constitutive activation/altered substrate.
      - Often recurrent - test for known mutations.
        - e.g., c-Kit; c-RAS; Ret, EGFR, etc.

**Mutations in GIST**

- **GIST:** CD117/PDGFRA positive GI stromal tumors.
  - c-kit mutations: constitutively activated KIT tyrosine kinase.
    - Juxtamembrane domain (exon11) or transmembrane domain (exon 9)
      - Imatinib (Gleevec) responsive.
    - Tyrosine kinase domain mutations:
      - Val654Ala, Thr670Ile: Imatinib resistant
EGFR mutations in Lung CA

- 10% of patients with Lung CA – rapid response to Gefitinib (Iressa)
  - Non smokers
  - Females
  - Japanese
  - Adenocarcinoma
- Mutations in exons 18, 19, &21
  - Kinase catalytic domain
  - Increased inhibition by Gefitinib

Minimal Residual Disease

- Quantitative determination of tumor-specific fusion transcripts.
  - Presence vs quantitation.
- Detection of clone-specific sequences for T and b-cell neoplasms.
  - (Problem: ongoing mutations in antigen receptor genes).

Minimal Residual Disease/ Molecular Staging

- Cell-type specific transcript (mRNA):
  - PSA (prostate);
  - mammaglobin (breast);
  - CEA in lymph nodes (adenoCA, e.g., Colon);
  - tyrosinase (melanoma);
  - thyroglobulin (thyroid).
  - Caveat: ? cell-type specificity of low copy-number transcripts.

MRD/Molecular Staging

- RT-PCR for mets in histo negative sentinel nodes.
- Melanoma: Tyrosinase, MART-1, MAGE, GalNAc-T, PAX3
  - Variable results; ? Increased recurrence in histo-/PCR+, vs. histo-/PCR-
- Breast: Mammaglobin1, mammaglobin 2, CEA, CK19, etc.

Tumor Classification/diagnosis W/ Microarrays

- Label total RNA from a tumor
- hybridize to chip w/ ≥ 25,000 cDNAs/oligonucleotides.
  - Expression profile unique to tumor type.
  - ? Predict behavior
  - ? Identify origin of mets
  - ? Identify targets for therapy.

Molecular Genetic Tests

- Genetic test:
  - Analysis of human
    - DNA
    - RNA
    - chromosomes
    - proteins
    - metabolites
  - to detect heritable disease-related
    - genotype,
    - phenotype
    - karyotype
  - for clinical purposes.
Genetic Diagnosis

"Purpose"

- Diagnostic Testing
- Screening
- Presymptomatic Testing
- Prenatal testing
- Preimplantation Diagnosis
- Pharmacogenetic testing
- Susceptibility to environmental agents

Genetic Alterations

- Chromosomal alterations
- "Gene-level" alterations.

Test Choice

- Cost
- Sample requirements
- Turnaround time
- Sensitivity/Specificity
- Positive/ Negative predictive value
- Type of mutation detected
- Genotyping vs mutation scanning

Conventional Cytogenetics (Karyotyping)

- Detect numerical structural chromosomal alterations
  - trisomy
  - monosomy
  - duplications
  - translocations, etc.

Conventional Cytogenetics (Karyotyping)

- Advantages:
  - evaluate all chromosomes
    - prior specification of chromosome unnecessary
    - detect unsuspected abnormality
    - detect balanced alterations
      - (No gain or loss of genetic material)
  - FISH may be performed.
    - characterize unexpected alterations

Conventional Cytogenetics (Karyotyping)

- Disadvantages:
  - Need for live cells to grow in culture
    - (ACMG standards, failure <1%).
  - Turnaround time - up to 10 days
    - (ACMG standards - 90% of results w/in 14 days)
  - Labor Intensive
FISH
• Use of fluorescently labeled probes to specifically visualize
  – entire chromosomes (chr. paint probes)
  – centromeres (centromeric probes)
  – specific loci (locus-specific probes)
• Metaphase
  – All types of probes
• Interphase
  – Centromeric and locus-specific probes only

FISH
• Identify:
  – translocations
  – marker chromosomes
  – Small deletions/duplications w/ locus-specific probes
  • e.g., DiGeorge's syndrome.

Interphase FISH
• rapid (<48 hours) detection of
  – Numerical abnormalities
  – Duplications/deletions/amplifications
  – translocations
  – mosaicism

Interphase FISH
• Prenatal Chr.13, 18, 21, X + Y
  – approx. 75-85% of all clinically relevant abnormalities.
• Dual color FISH w/ subtelomeric probes:
  – Prenatal dx of chromosomal translocations

Interphase FISH
• Need for confirmatory conventional cytogenetic testing.
• Need to specify chromosome
  – Information only about specific chromosome/locus tested.

Metaphase FISH
• Supplement conventional cytogenetics
  – Identify marker chromosomes
  – extra unknown material attached to chromosome/loss of segment
  – detect/identify rearrangements (incl. cryptic translocations),
  – identify/quantify mosaicism
### Metaphase FISH
- Need to specify Chromosome/locus
  - Multiple tests to identify marker chromosome.
  - Multiprobe FISH.

### Gene Dosage
#### Gains/Losses
- Comparative genomic hybridization (CGH)
  - Label normal and test DNA with separate dyes
  - Competetively hybridize to
    - Metaphase Spread or
    - cDNA array.
  - Detect Gains and losses.

### Gene Dosage
#### Gains/Losses
- Classical CGH
  - Hybridize to metaphase spread
    - Resolution approximately 5Mb
  - Information on all chromosomes
  - No need for culture.
    - Can use archival material (e.g., placenta, POC, tumor etc.)
  - Single cell DNA amplification & CHG
    - Applicable to preimplantation genetic diagnosis (PGD)

### Gene Dosage
#### Gains/Losses
- Array-based CGH
  - Hybridize to BAC-based or cDNA array.
  - Higher resolution (50kb vs 5MB)

### Gene Dosage
#### Gains/Losses
- PCR-based methods
  - Real-time (quantitative) PCR.
  - Microsatellite PCR.
  - Long-range PCR.
  - Probe amplification techniques.
- Rapid
- For specific loci
  - May be “multiplexed” for multiple loci

### Molecular Tests
- Test for:
  - Karyotype
  - Gain or loss of genetic material ("dosage")
  - Genetic linkage
  - Known/recurrent mutations
  - Variations in lengths of repeat sequences
  - Alterations in DNA methylation
  - Unknown mutations in multiple genetic segments
Types of mutations-gene

- **Point mutations**
  - Missense (change an amino acid)
  - Nonsense (premature termination)
  - Silent
- **Deletion**
  - Large variation in size
- **Insertion**
- **Duplication**
- **Splice site**
- **Regulatory**
- **Expanded repeat**

### Point Mutations

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>ATC</td>
<td>TTC</td>
<td>AGC</td>
<td>TGC</td>
<td>GAG</td>
<td>CTA</td>
<td>TAT</td>
</tr>
<tr>
<td>Leu</td>
<td>Phe</td>
<td>Ser</td>
<td>Cys</td>
<td>Glu</td>
<td>Leu</td>
<td>Tyr</td>
</tr>
</tbody>
</table>

### Missense Mutations

- Change Amino Acid
- Change Protein Structure/function
  - Depending upon specific AA change
- Loss of function:
  - e.g., Hb S (GAG to GTG – Glu to Val),
    Hemochromatosis (C282Y)
- "Gain of Function":
  - e.g., Factor V Leiden
- No functional effect:
  - e.g., KVLQT1 P448R

### Missense mutations

- When is a missense mutation significant?
  - known structural and functional domain
  - evolutionarily conserved residue
  - independent occurrence in unrelated patients
  - absent in large control sample
  - novel appearance & cosegregation w/disease phenotype in pedigree
  - in vitro loss of function
  - restoration of function by WT protein.

### Nonsense Mutations

- Amino Acid codon to "Stop"
- Three stop codons
  - UAA, UAG, UGA
- Truncated protein
  - Protein truncation test
- E.g., Beta0 Thalassemia in Sardinia
  - Codon 24, CAG to TAG
Deletions

- Complete/partial gene deletion
  - Duchenne Muscular Dystrophy
  - Alpha thalassemia
- Multiple genes ("contiguous gene syndromes"
  - DiGeorge Syndrome
  - TSC2-PKD1
  - WAGR syndrome

Insertions

- Tay Sachs Disease
  - 4bp insertion in Ashkenazi Jews
- Hemophilia A
  - L1 insertion in FVIII gene (1% of patients)

Splice junction mutations

- GT/AG rule
  - AAGGTGTAGT. . . . . / . . . . YYYYYYYYYNCAAG
- Loss of splice site
  - intron not spliced out
- Creation of novel splice sites
  - >100 mutations
    - e.g., Hemoglobin E
      - Missense mutation and splice site error
      - Both normal and new splice site use
        - Hemoglobinopathy AND thalassemia features

Frame-shift Mutations

- Codon = 3 bp
- insertion/deletion not multiple of 3bp
  - Change of reading frame - entire protein altered.
  - e.g., Tay Sachs 4 bp insertion, BRCA1 185 delAG, BRCA2 6174delT, etc.
  - blood group O (1 bp deletion)

Other mutations

- Cap-site Mutants
- Mutations in initiation codons
- Creation of a new initiation codon
- Mutations in termination codons
- Polyadenylation/cleavage signal mutations.
Unstable trinucleotide repeats

- Fragile X Syndrome (CGG)n 5'UT
- Huntington’s syndrome (CAG)n polyglutamine
- Myotonic dystrophy (CTG)n 3'UT
- SCA type 1 (CAG)n polyglutamine
- Friedrich’s Ataxia (GAA)n intron 1

Mutation Testing

- Tests for recurrent mutations.
  - Limited # of specific mutations.
  - significant proportion of cases e.g., Factor V Leiden, Hemochromatosis.
- Mutation Scanning Methods.
  - Multiple "private" mutations of one or more genes.
  - e.g., BMPR2 mutations in familial PPH,
- Combination.
  - e.g., BRCA1/2, CFTR etc.

Recurrent Mutation Tests

- Many rapid methods.
- High sensitivity/specificity.
- Test choice - laboratory preference
  - Workflow, equipment, kit availability
  - patent issues, etc.
- Detect
  - heterozygotes,
  - compound heterozygotes
  - homozygotes

Recurrent Mutation Tests

- Choice of mutation tested
  - Clinical syndrome
  - Family history
  - Ethnicity
- Positive results
  - Unambiguous
  - Technical false positive rare (most methods)
  - Positive predictive value, penetrance, etc. usu known

Recurrent Mutation Tests

- Negative predictive value:
  - Population screening:
    - 1 - (ethnic prevalence x [1 - sensitivity for specific ethnic group])
  - Family history (index case w/ unknown mut)
    - 1 - (prior probability x [1 - sensitivity for specific ethnic group])
  - Family history (known mutation in index case)
    - 100%
  - Affected individual (unknown mutation)
    - 0%

Recurrent Mutations

- Methods
  - PCR-RFLP
  - Allele-specific probes/primers
  - Direct sequencing/"Minisequencing"/Pyrosequencing.
  - Molecular Beacons/TaqMan probes.
  - Oligonucleotide ligation assay.
  - Mass spectroscopy-based methods.
PCR-RFLP
- Quick, Robust
- Sources of error:
  - Amplification failure of one allele
    - linkage disequilibrium with primer site polymorphism (HFE)
  - Failure of restriction enzyme
  - Control in same tube
  - Different variants with loss of same restriction site

Real-Time PCR
- New instruments can monitor PCR during thermocycling
  - intercalating dye:
    - non-specific increase in fluorescence with increased double-stranded DNA
    - "Melting curve" analysis - monitor denaturation of double-stranded DNA
  - Probes using Fluorescence Resonance Energy Transfer ("FRET")
    - Monitor binding of probe to wild-type or mutant allele.

Repeat Expansions
- Southern Blotting Methods
  - Gold Standard
  - Labor intensive
  - need for high quality DNA
- PCR-based Methods
  - Rapid
  - Amplification failure with very long repeats.

Expanded Repeats-Huntington Disease

<table>
<thead>
<tr>
<th>[CAG]_{10-26}</th>
<th>[CAG]_{27-35}</th>
<th>[CAG]_{35-41}</th>
<th>[CAG]_{42-121}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>At risk for expansion</td>
<td>Variable penetrance</td>
<td>Affected</td>
</tr>
</tbody>
</table>

Mutation Scanning Methods
- Test one or more genes for unknown variation in.
  - Exons
  - Introns
  - splice sites
  - Promoters/enhancers
  - "locus control region"

Mutation Scanning Methods
- Ideal method:
  - Screen large DNA sequence
  - 100% sensitivity and specificity
  - Unambiguously define mutation.
  - Minimum # of steps
  - High throughput
  - No special equipment
  - No dangerous reagents
- No such method
  - Compromise
### Screening Methods

- Physical properties of amplified gene segments
  - Denaturation profile, electrophoretic mobility, etc.
  - SSCP
  - DGGE
  - DHPLC
  - Cleavage fragment length polymorphisms
  - Heteroduplex analysis
  - Dideoxy fingerprinting.

### Screening methods

- Sensitivity determined by specific mutation
- Need for multiple conditions
- One datapoint per gene segment evaluated
- Screen for presence, not identity of mutation.

---

### Mutation Scanning Methods

- **Direct Sequencing**
  - Screen presence and identity of mutation
  - Bidirectional sequencing
  - 2 data-points per base sequenced.
  - DNA sequencing
    - Usu. multiple exons tested.
    - Splice-site mutations may be missed, especially mutations deep in large introns.
  - RNA sequencing
    - Need for cells w/c express gene
    - "Nonsense mediated decay"
    - RNA more labile

### Direct Sequencing Methods

- Automated fluorescent sequencing
  - DNA/cDNA amplification, purification, and re-amplification with fluorescent "Big-Dye" terminators.
  - Widely available
  - Need to visually scan electropherograms
    - Verify "base calling", heterozygous bases

### Direct Sequencing Methods

- Pyrosequencing
  - Limited to short sequences.
  - Need to optimize algorithm for each segment
- Chip-based" sequencing
  - Rapid
  - Reduced sensitivity for heterozygous and frame-shift mutations.

### Interpretation of Variant

- Previously reported variant
  - Known to be cause of disorder
  - Known to be "neutral variation"
Interpretation of Variant

• New variant:
  – Type likely to be assoc. w/disorder
    • frame-shift mutation
    • start “ATG” mutation
    • “Stop codon”
    • splice-junction mutation
    • non-conservative missense in active site,

• New Variant
  – Type likely to be “neutral”
    • e.g., no change in amino acid, and not
      cryptic splice site
  – Type w/c may or may not be assoc. w/ disorder
    • E.g., non-conservative missense
      mutation, in region not known to be
      active site, etc

Interpretation of Variant

• Recessive Disorders.
  – Test parents to ensure two variants in
    trans (separate alleles) not in cis
    (same allele).

Testing Strategies.

• Single gene disease w/ only recurrent
  mutations (e.g. Achondroplasia or
  MEN2)
  – Test for recurrent mutation
  – Positive result
    • penetrance known
  – Negative result
    • False negative rate known.
    • Phenotypic testing, if indicated.

Testing Strategies.

• “Single gene” condition w/ repeat
  polymorphisms (Fragile X)
  – Test for repeat polymorphisms using either
    • Southern Blotting
    • PCR (very large expansions may be
      missed)
  • Clinical syndrome w/ multiple genes
    • “recurrent” (SCA)
    • Private (Long QT)
Testing Strategies

Cystic Fibrosis

<table>
<thead>
<tr>
<th>CFTR Screening</th>
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</thead>
<tbody>
<tr>
<td>• Carrier frequency in various ethnic populations</td>
</tr>
<tr>
<td>- European Caucasian:  1/25</td>
</tr>
<tr>
<td>- Ashkenazi Jewish:    1/25</td>
</tr>
<tr>
<td>- Hispanic American:   1/46</td>
</tr>
<tr>
<td>- African American:    1/65</td>
</tr>
<tr>
<td>- Asian American:      1/90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CFTR Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>• CFTR Gene:</td>
</tr>
<tr>
<td>- 250 kb</td>
</tr>
<tr>
<td>- 27 Exons</td>
</tr>
<tr>
<td>- 6.5kb mRNA</td>
</tr>
<tr>
<td>- In-frame deletion of codon 508 in 70% of cases (Caucasians/Ashkenazim)</td>
</tr>
<tr>
<td>-&gt;1000 mutations reported</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>CFTR Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ACMG recommendations</td>
</tr>
<tr>
<td>- Testing offered to all Caucasians and Ashkenazim, made available to all other ethnic groups</td>
</tr>
<tr>
<td>- Simultaneous or sequential couple screening</td>
</tr>
<tr>
<td>• Give results to both partners</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CFTR Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal pan-ethnic core mutation panel consisting of:</td>
</tr>
<tr>
<td>- 25 mutations.</td>
</tr>
<tr>
<td>- 3 exonic polymorphisms as reflex tests.</td>
</tr>
<tr>
<td>- 5/7/9T intronic polymorphism as reflex test only if R117H is positive.</td>
</tr>
</tbody>
</table>
CFTR Screening
- Extended mutation panels for positive-negative couples not encouraged
- Reporting of results and residual risks should be based on model report forms developed by ACMG committee
- Primary care providers uncomfortable w/ these complexities should refer pt to genetic counselor

CFTR Screening
- 5T/7T/9T intronic polymorphism
- R117H + 5T in cis - CF
- R117H + 7T in cis - CBAVD
- R117H (etc.) + 5T in trans – CBAVD
- 5T/5T homozygosity - CBAVD
  - R117H causes CF only when w/ 5T on same allele
  - 5T with least efficiency of RNA processing
  - 5T in 5% of US population

CFTR Screening
- Limitations
  - Inability to detect all CF mutations
  - Correct paternity assumed; results applicable only for current reproductive partners
  - Assumes family history is truly negative
  - Poor genotype-phenotype correlation - prognostic prediction in affected offspring difficult

CFTR Screening
- Concurrent testing: Both partners screened, both informed.
- Advantages:
  - Quicker
  - Alerts both partners w/ current and future partners
  - Informs both families of potential risk
- Disadvantage:
  - Anxiety
  - Cost

CFTR: INCIDENCE, CARRIER, MUTATION RATES: BY POPULATION

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th>Carrier freq.</th>
<th>%ΔF508</th>
<th>% other &quot;common&quot;</th>
<th>% group-specific</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>1:3,300</td>
<td>1/29</td>
<td>70</td>
<td>13</td>
<td>80-90%</td>
<td>97%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1/8-9000</td>
<td>1/46</td>
<td>46</td>
<td>11</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>Ashkenazi</td>
<td>1:3,300</td>
<td>1/29</td>
<td>80</td>
<td>67</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>Native Am</td>
<td>1:1500-3970</td>
<td>0</td>
<td>25</td>
<td>69</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>African Am</td>
<td>1:15,300</td>
<td>1:60-65</td>
<td>48</td>
<td>23</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>Asian Am</td>
<td>1:32,100</td>
<td>1:90</td>
<td>80</td>
<td>0</td>
<td>30%</td>
<td></td>
</tr>
</tbody>
</table>
Negative results: + family history

• Caucasian Couple
  • each w/ sibling with CF.
    – (Prior Probability of each parent being a carrier = 2/3).
• Both test negative for the 25 mutations.
  – Probability parent is carrier = \((0.67 \times (1 - 0.9)) = 0.067\).
  – Probability both parents carriers = .004489
  – Probability of affected child = 1 in 900

• Hispanic Couple w/ same history and results:
  – Probability of being carrier = \((0.67 \times (1 - 0.57)) = 0.287\);
  – probability of an affected child = 1 in 48!
  – (versus untested prob.: .67*.67*.25=1/9)

• Asian couple w/ same hx and results:
  – probability of affected child 1 in 18!

Genetic testing additional considerations:

• Benefits Vs. Risk of Testing:
  – Availability of treatment/prevention of clinical syndrome
  – Presence or absence of pre-clinical manifestations.
  – Discrimination:
    • Insurance
    • Employment
    • Confidentiality

Additional Considerations

• Potential interventions:
  – Behavioral
    • lung cancer-risk - smoking cessation;
    • heart disease risk - diet/exercise;
    • risk of breast/colon cancer - screening acceptance.
  – Medical
    • e.g., prophylactic mastectomy/thyroidectomy;
    • blood-letting/blood donation for HFE;
    • anti-arrhythmics for Long QT, etc.

• Pre-morbid/clinical syndrome
  – Is there a clinically identifiable syndrome?
  – ? Need for intervention prior to clinical manifestations

• Technical considerations
  – e.g., Fragile X-syndrome.

• Patent issues
  – affect availability/cost of testing
### Additional Considerations

- **Ethics**
  - Implications for patients and relatives.
  - E.g., identical twins; siblings;
  - Paternity issues -
- **Legal issues**
  - New York State Civil Right Law:
    - Need for informed consent
    - Genetic testing only (not phenotypic testing)
    - Standards for informed consent in civil rights law, section 79-l
    [http://assembly.state.ny.us/leg/?cl=17&a=12].

### Factors affecting utility of genetic testing

- **Increased utility**
  - High morbidity and mortality of the disease
  - Effective but imperfect treatment
  - High predictive power of genetic test (high penetrance)
  - High cost or onerous nature of screening and surveillance methods
  - Preventive measures that are expensive or associated with adverse effects

- **Decreased utility**
  - Low morbidity and mortality of disease
  - Highly effective and acceptable treatment (i.e., no harm is done by waiting for clinical disease to treat patient)
  - Poor predictive power of the genetic test (low penetrance)
  - Availability of inexpensive, acceptable, and effective surveillance methods (or need for surveillance whether or not one has increased genetic risk)
  - Preventive measures that are inexpensive, efficacious, and highly acceptable - e.g., folate supplementation.

### Ordering Molecular Tests

- **Patient preparation:** None
  - Avoid heparin: interferes with PCR.
- **Specimens:**
  - Fresh whole blood: EDTA/Citrate
  - Fresh tissues
  - Frozen tissues
  - Paraffin embedded tissues
  - Slides etc.

- **Specimen Handling**
  - DNA-based tests:
    - Room temperature, up to 72 hours (maybe more with special buffers)
  - RNA-based tests:
    - Deliver ASAP (4-6 hours)
    - Special considerations for proprietary test.

### Essential info (Molecular Genetic Tests):

- Clinical information
- Pedigree, if possible
- Race
- Reason for testing.

### Informed consent:

- New York State Civil Rights Law.
  - Nature of test; availability of genetic counseling; implications of positive and negative tests, etc.